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Determination of Total, Viable Cells and *Enterobacteraceae* in Categorized Milk Powder

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Abstract: The present study was conducted to examine the microbiological quality of commercial milk powders. A total of 30 dried milk powders, 10 each of Skim Milk Powder (SMP), Semi Skim Milk Powder (SSMP) and Full Cream Milk Powder (FCMP) purchased from market of Hyderabad, Sindh were evaluated for microbiological quality characteristics, like Total Viable Count (TVC), thermoduric count and *Enterobacteraceae* Count (EbC). Total viable count, ($6.1 \times 10^3 \pm 7.2 \times 10^2$cfu/g) and *Enterobacteraceae* count, ($2.3 \times 10^3 \pm 2.6 \times 10^2$cfu/g) were significantly ($p<0.05$) higher in FCMP compared to SMP ($3.7 \times 10^3 \pm 9.8 \times 10^2$ and $1.7 \times 10^3 \pm 1.7 \times 10^2$cfu/g, respectively) and SSMP ($3.5 \times 10^3 \pm 4.4 \times 10^2$ and $1.5 \times 10^3 \pm 1.0 \times 10^2$cfu/g, respectively). The overall average concentration of TVC ($4.43 \times 10^3 \pm 4.8 \times 10^2$cfu/g) in dried milk powder was recovered lower (11.28 folds) compared to Pakistan Standard Institution (PSI) and/or Indian Standard Institution (ISI) (5.0×10^4 cfu/g) and the overall average count of Eb ($1.84 \times 10^3 \pm 1.2 \times 10^2$) were detected higher, (18.4 folds) compared to ISI standard respectively. Although TV count were within the range of standard of specification (PSI/ISI), but the counts of, Eb indicates the unhygienic condition of dried milk powders with higher risk level for human health.

Key words: Milk powders, skim milk powder, semi skim milk powder

INTRODUCTION

Milk is a major part of food consumption and plays a prominent role in the Pakistani diet and comes second to cereals in the level of per capita consumption (Anonymous, 2008). No doubt, the milk powders are generally considered as product of good microbiological quality with no risk of spoilage, but several factors may contribute to change its physical and chemical properties which reduce shelf-life and thus its commercial value (Cousins *et al*., 1987). Although the micro-organisms in dried milks cannot grow due to its low moisture content and do not play any direct role in their spoilage. But their occurrence in these products is of great significance and serves as an index of hygienic standards maintained during production, processing and handling. As these powders find their application in dairy industry, for processed cheese or for reconstitution purposes, the presence of micro organisms even in low numbers may cause potential hazards and/or defects in the derived products (Yadav *et al*., 1993). The milk provides a highly nutritious substrate that can support the wide variety of bacteria as well as yeast and molds for their growth and reproduction (Phillips and Griffiths, 1990). The contamination role of bacteria during the production of milk powder has been well documented. With this many studies have provided evidence of vegetative growth during the manufacturing of milk powders. The other contamination source in milk powders are reuse of by products such as buttermilk and permeate from milk ultra filtration ingredients added to the process such as lactose and recycle loops in manufacturing plants (Hill and Smythe, 2004). As these powders find their utilization in dairy sector, for yoghurt, tea, ice-cream and cheese making or for reconstitution purposes, the presence of micro organisms even in low numbers may cause potential hazards and/or defects in the derived products (Yadav *et al*., 1993). Since, still no work has been found on any aspects of milk powders in the province of Sindh. Thus, present study has been designed for determination evaluation quality of milk powders.

MATERIALS AND METHODS

Collection of milk powder samples: A total of thirty samples of milk powders i.e 10 from each category (skim, semi skim and full cream) were collected in a sterilized sample bottles from the randomly selected milk powder shops of Hyderabad and brought to the Laboratory of Dairy Microbiology, Department of Dairy Technology. Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, for the evaluation of microbial quality characteristics. However, among the thirty samples, six samples showed either spreaded colonies and/or heavily contaminated. Thus rejected and not included in the present study.

Preparation of diluent

Peptone water: Peptone water (Oxoid, Ltd. England) (15 g) was dissolved in distilled water (1 L) and distributed in bottle (90 ml) and/or in test tubes (9 ml). The

bottles/tubes were capped or plugged with cotton and autoclaved (121°C) for 15 min. The sterilized diluents were stored at (4-8°C) till use.

Preparation of test samples: Milk powder (10 g) was diluted in warm (45°C) sterile diluents I-e peptone water solution (90 ml) to make primary dilution (10^{-1}). Then a series up to 10^{-5} dilution was prepared by transferring primary dilution (1 ml) into test tube containing sterile diluents (9 ml) to obtain 10^{-2} dilution and repeating the operations with sterile diluents (9 ml) using the 10^{-2} and further dilutions to obtain 10^{-3}, 10^{-4} and/or 10^{-5}.

Enumeration of total viable count (colony count technique at 30°C): Total viable counts were enumerated according to the method of International Dairy Federation (IDF, 1991). Pre prepared test sample (1ml) of 10^{-3}, 10^{-4} and/or 10^{-5} dilutions (section-3.9.1) was transferred into sterile petri dishes in duplicate through sterile graduate pipette and/or dispensing pipette (1000 µl) with sterile plastic tips and warm (45±1°C) sterile plate count agar medium (15 ml) was mixed with inoculum. The mixture was allowed to solidify and incubated (30°C) for 72±2 h.

Enumeration of *Enterobacteraceae* counts (colony count technique at 37°C): *Enterobacteraceae* counts were enumerated according to the method of British Standard Institute (BSI, 1993). Pre prepared test sample (1 ml) of 10^{-1}, 10^{-2} and/or 10^{-3} dilution (section 3.9.1) was transferred into sterile petri dishes through dispensing pipette (1000 µl) with sterile plastic tips and warm (45±1°C) sterile violet red bile agar medium (15 ml) was mixed with inoculum. The mixture was allowed to solidify and incubated (37°C) for 24±2 h.

$$N=\frac{\sum c}{(n_1 + 0.1x\, n_2)\, d}$$

N: Number of colonies obtained
$\sum c$: Sum of colonies counted on all the dishes retained
n_1: Number of dishes retained in the first dilution
n_2: Number of dishes retained in the second dilution
d: Dilution factor corresponding to the first dilution

RESULTS

Total Viable Count (TVC): Total viable count of Skim Milk Powder (SMP) Semi Skim Milk Powder (SSMP) and Full Cream Milk Powder (FCMP) was evaluated and the results are presented in Fig. 1. A wide variation was observed in TV counts in all types of dried milk powders examined in the present study. The concentration of TV count in SMP, ranged between 1.2 x 10^3 to 9.2 x 10^3 cfu/g and averaged 3.7 x 10^3±9.83 x 10^2 cfu/g. While in case of SSMP, the TV counts were observed in between 1.8 x 10^3 to 5.3 x 10^3 cfu/g with mean value of 3.5 x 10^3±4.36 x 10^2 cfu/g. Where ever, TV count in FCMP, varied between 2.9 x 10^3 to 9.6 x 10^3 cfu/g and averaged 6.1 x 10^3±7.23 x 10^2 cfu/g. Moreover, the results of statistical analysis (Analysis of Variance) showed significant difference ($p<0.05$), in TV counts in SMP, SSMP and FCMP (Appendix-II). It was further observed that TV count of FCMP (6.1 x 10^3±7.23 x 10^2 cfu/g) was significantly ($p<0.05$). While there was no significant difference ($p>0.05$) in TV counts observed between SMP and SSMP. The concentration of TV counts was lower in SMP (13.55 folds), SSMP (14.24 folds) and in FCMP (8.21 folds) compared to Indian Standards Institute, ISI and Pakistan Standard Institution I-e ≤ 5.0 x 10^4 cfu/g. While the overall concentration of TV counts of all types of milk powders were lower (2.25 folds) compared to ISI standard ≤ 5.0 x 10^4 cfu/g. While the overall average concentration of TV counts in FCMP, SSMP and SMP was (11.28 folds) lower than PSI/ISI standard 5.0 x 10^4 cfu/g (Table 1).

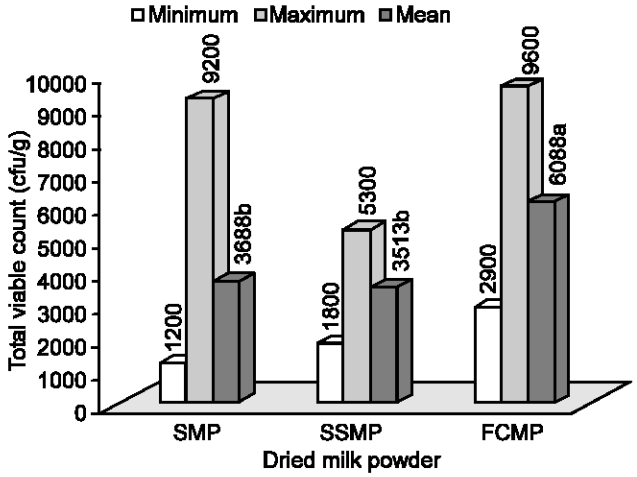

Fig. 1: Graph shows minimum, maximum and mean values of total viable counts (cfu/g) in skim milk, semi skim milk and full cream milk powders

SE± = 1058
LSD (0.05) = 2201
SMP = Skim Milk Powder
SSMP = Semi Skim Milk Powder
FCMP = Full Cream Milk Powder
cfu = Colony Forming Unit

***Enterobacteraceae* Count (EBC):** SMP, SSMP and FCMP were evaluated for *Enterobacteraceae* count and the results are depicted in Fig. 2 and Appendix-VII. TPS counts varied greatly in all types of dried milk powders examined in the present study. The concentration of *Enterobacteraceae* count in SMP ranged between 1.1 x 10^3 to 2.6 x 10^3 cfu/g and averaged 1.7 x 10^3±1.6 x 10^2 cfu/g. While in case of SSMP the *Enterobacteraceae* counts were observed in between 1.1 x 10^3 to 1.9 x 10^3

Table 1: Total Viable Counts (cfu/g) in different dried milk samples compared to ISI and PSI standards

	Total viable count (TVC) cfu/g	
Sample	Observed (a)	Deviation in folds from PSI/ISI standard (b) = (x) ÷ (a)
SMP	3690	-2.71
SSMP	3510	-2.84
FCMP	6090	-1.64
Mean	4430	-2.25

a = Observed Values, x = (Standard Value of PSI/ISI = ≤50000 cfu/g), ISI = Indian Standards Institute, PSI = Pakistan Standard Institute

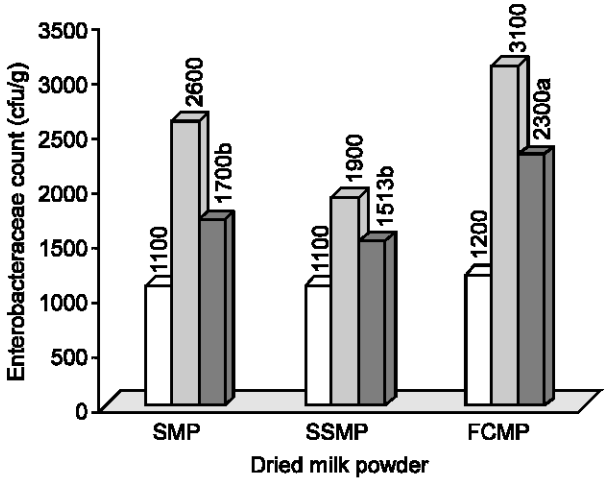

Fig. 2: Graph shows minimum, maximum and mean values of *Enterobacteraceae* count (cfu/g) in skim milk, semi skim milk and full cream milk powders

SE± = 267
LSD (0.05) = 554
SMP = Skim Milk Powder
SSMP = Semi Skim Milk Powder
FCMP = Full Cream Milk Powder
cfu = Colony Forming Unit

and averaged 1.51 x 10^3 ± 1.03 x 10^2 cfu/g. *Enterobacteraceae* count in FCMP, varied between 1.2 x 10^3 to 3.1 x 10^3 cfu/g and averaged 2.3 x 10^3 ±2.60 x 10^2cfu/g. Moreover, the results of statistical analysis (AOV) showed significant difference ($p<0.05$), in *Enterobacteraceae* counts in SMP, SSMP and FCMP (Appendix-VIII). Further LSD comparison of means (0.05) observed that *Enterobacteraceae* b count of FCMP (2.3 x 10^3±2.6 x 10^2 cfu/g) was significantly ($p<0.05$), higher than SMP (1.7 x 10^3±1.6 x 10^2 cfu/g). SSMP (1.51 x 10^3 ±1.03 x 10^2 cfu/g). While there was no significant difference ($p>0.05$), in *Enterobacteraceae* counts observed between SMP and SSMP), higher than SMP (3.7 x 10^3±9.83 x 10^2 cfu/g) and SSMP (3.5 x 10^3±4.36 x 10^2 cfu/g). The concentration of *Enterobacteraceae* counts was higher in SMP (17 folds), SSMP (15.1 folds) and in FCMP (23 folds) compared to that of Indian Standards Institute (ISI, 1975) I-e ≤ 1.0 x 10^2 cfu/g. While the average concentration of *Enterobacteraceae* counts of all types of milk powders were observed (18.4 folds) higher in contrast to ISI standard ≤1.0 x 10^2 cfu/g (Table 2).

Table 2: *Enterobacteraceae* counts (cfu/g) in different dried milk samples compared to ISI standards

	Enterobacteraceae Count (EbC cfu/g)	
Sample	Observed (a)	Deviation in folds (b) ÷ (a)
SMP	1700	+17
SSMP	1510	+15.0
FCMP	2300	+23
Mean	1840	+18.4

a = Observed Values, x = (Standard Value of ISI (1975) = ≤1.0 x 10^2 cfu/g), ISI = Indian Standards Institute

DISCUSSION

Present study has been conducted to assess the general hygienic quality of dried milks (full fat, low fat and fat free milk powders) and the extent of microbes have been observed. Although the microorganisms in dried milk owing to their low moisture content can not grow and thus do not play any direct role in their spoilage, their occurrence in these products is of great significance they server as an index of hygienic standards maintained during Production, Processing and handling (Yadav *et al*., 1993). However, in dairy industry, these powders have significant use in the production of dahi, yoghurt, ice-cream, tea and/or reconstitution purpose and presence of microorganisms may cause defects in the derived products. In the present study the total viable count of FCMP (6.09 x 10^3±7.23 x 10^2 cfu/g) was significantly ($p<0.05$) higher than SMP (3.69 x 10^3±9.83 x 10^2 cfu/g) and SSMP (3.51 x 10^3±4.36 x 10^2 cfu/g). While, there was no significant difference ($p>0.05$) in TV counts observed between SMP and SSMP. It is of interest to point out that total viable count (4.43 x 10^3±4.8 x 10^2 cfu/g) observed in the dried milk is lower than reported by (Rueckert *et al*., 2005; Khaskheli, 1998; ISI, 1975 and PSI, 1982) i.e $5.6x10^4$±$4.3x10^3$, $8.7x10^3$, $1.0x10^4$ and $5.0x10^4$ respectively. The vegetative cells normally killed at 80°C for 10 mins. But the present study manifested their presence in milk powders. Because, their ability to attach with stainless steel and folded surface (Flint *et al*., 2006). Once they attach to the surface, vegetative cells grow with spores by forming biofilms. This biofilms is not completely removable by CIP system but can be decreased; the remaining contaminants present on folded stainless steel transfer into final product, i.e powders milk (Parker *et al*., 2001).

Enterobacteraceae count of FCMP ($2.3 \times 10^3 \pm 2.60 \times 10^2$ cfu/g) was significantly ($p<0.05$) higher than SMP ($1.7 \times 10^3 \pm 1.69 \times 10^2$ cfu/g) and SSMP ($1.51 \times 10^3 \pm 1.03 \times 10^2$ cfu/g). While there was no significant difference ($p>0.05$), in *Enterobacteraceae* counts observed between SMP and SSMP. The mean value ($1.84 \times 10^3 \pm 1.25 \times 10^2$) obtained in present study is lower than reported by Taha *et al.* (1972) i.e 13×10^8. It is the general concept that *Enterobacteraceae* are not present in powder milk because they grow at 30-37°C and can not survive during processing, it is true hypothesis and proved by various researches. But, mostly *Enterobacteraceae*. The powder milk is packed hygienically in large sterilized containers and bags. However, transportation some damaged containers and bags have been observed, probably they can contaminate the milk powders. When milk powder reaches up to whole seller it has been re-packed in and/or small bags and containers which are not sterilize. Further the retailers decrease the size of the bags 1 Kg and/or ½ Kg to sell the consumers. During these practices no measures of hygienic conditions are adopted which may increase the *Enterobacteraceae* concentration in powder milk.

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